STANFORD UNIVERSITY LIBRARY
APR 21 1931

THE EFFECT OF INADEQUATE RATIONS ON THE COMPOSITION OF THE BLOOD AND OF THE BONE OF CHICKS

by

L. C.

CHARLES LESLIE SHREWSBURY, A. B.

Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI

1928

STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

3781
M67S
UNCLASSED

THE EFFECT OF INADEQUATE RATIONS ON THE COMPOSITION OF THE BLOOD AND OF THE BONE OF CHICKS

by

CHARLES LESLIE SHREWSBURY, A. B.

Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI

1928

Digitized by the Internet Archive
in 2025

https://archive.org/details/effectofinadequa0000char

The Effect of Inadequate Rations on the Composition of the Blood and of the Bone of Chicks

Albert G. Hogan, Charles L. Shrewsbury, and Harry L. Kempster

ABSTRACT.—Chicks reared on synthetic diets usually develop symptoms of a deficiency disease. In an effort to define more precisely the nature of the deficiency, chicks reared on such rations were used for the analysis of blood and bone. These analyses were then compared with similar determinations made on normal chicks, and on chicks receiving rations deficient in one of the following vitamins, A, B, or D. The analyses of chicks receiving rations deficient in vitamin A were normal. A ration deficient in vitamin B apparently raised the level of blood sugar. A deficiency of Vitamin D lowered the ash content of the bones. The blood sugar of chicks reared on synthetic diets was high so we have adopted the tentative hypothesis that these rations are deficient in one or more factors of the vitamin B complex.

It has been demonstrated[1] that synthetic diets of the type commonly used are not adequate for normal growth of the chick. The rate of growth is retarded, and abnormalities, loosely classified as leg weakness, are common. The symptoms are variable. In some instances they are characterized chiefly by head retraction, in others the legs are rigidly flexed, and the chicks are unable to stand. Frequently complete relief is afforded by treatment with yeast, or some other carrier of vitamin B. Strangely enough, however, chicks reared on rations that were strongly fortified with vitamin B carriers also collapsed in the manner just described. Milk, and the Osborne-Wakeman concentrate[2], are conspicuous examples of such supplements. Whatever the explanation may be, if speedy relief is not obtained the chicks almost invariably pass into a state of coma and die. More rarely the symptoms are less severe, marked chiefly by deformity of one or both legs. These chicks seem quite normal otherwise and the rate of growth may be unaffected.

Under precisely the same environmental conditions we are able to rear chicks with complete success, on rations of natural foodstuffs. It seems certain, therefore, that the chicks suffer from a nutritional inadequacy, and we have attempted to determine more specifically what factor, or factors, may be responsible. We first attempted to show whether or not the deficiency we are concerned with can be identified with any known deficiency disease. With this possibility in mind, various dietary adjustments were made, in an effort to provide larger quantities

or better proportions of the now recognized nutrients. Some improvement resulted, but the problem was not solved. Our next step was to make analyses of the blood and of the bone of chicks suffering from the symptoms we have just described. These analyses were then compared with similar determinations made on normal chicks, and on chicks suffering from known deficiency diseases, caused by a lack of vitamins A, B, or D.

The methods of blood and bone analyses have been applied to various dietary diseases, and we are reviewing those that are most pertinent at this time. Only scattered attempts have been made at applying analytical procedures to animals suffering from a lack of vitamin A, and so far as we are aware, such studies do not include the class aves.

In studies of polyneuritis of birds, the method of blood analysis has been used frequently. Thus the view has been expressed that the spasms of acute polyneuritis are due to a lowered blood calcium caused by a dysfunction of the parathyroid gland Ungar[3] was unable to find evidence to support such a belief. The blood calcium of normal pigeons was reported by him as 10.04 mgm. per cent, while neuritic pigeons in acute spasms,had a blood calcium of 9.66 mgm. per cent. The difference was not regarded as significant.

Special attention has been given blood sugar values, since many investigators believe vitamin B is concerned with carbohydrate metabolism. Funk and v. Schonborn[4] were among the first to investigate that question. They reported blood sugar values of normal birds to be 80 to 140 mgm. per cent, while in pigeons on a diet free of vitamin B these values were markedly increased, in two instances to 350 mgm. per cent. In a later paper[5] Funk again reported marked differences. The mean blood sugar level of normal pigeons was 242 mgm. per cent, but in pigeons suffering from polyneuritis this value rose to a mean of 279. A group of Japanese writers[6] noted a rise in blood sugar, a decrease in the red cell and an increase in the white cell counts, and occasionally an acidosis in pigeons suffering from polyneuritis. Redenbough[7] made somewhat similar observations, and force-fed his pigeons to prevent starvation. According to him, normal birds averaged 195 mgm. per cent. of blood sugar, while neuritic birds averaged 368 mgm. per cent. Randoin and Lelesz[8] reported a normal level of 200 mgm. per cent of sugar in the blood, and then a gradual rise as polyneuritis developed to a final level of 274 to 282 mgm. per cent. Barlow[9] reported hyperglycemia, also anemia, in pigeons reared on diets free of vitamin B.

In contrast to the preceding reports Collazo[10] decided that the starving pigeon presents the same blood picture as the neuritic pigeon, and concluded that the rise is due to inanition. Kon and Drummond[11] came to the same conclusion. They reported that the slight hypergly-

cemia, as compared to the normal bird, is due to starvation and not to a lack of vitamin B. These studies were extended by Marrian, Baker, Drummond, and Woollard[12], and they reaffirmed the slight effect of a vitamin B deficiency on the blood sugar content. Thompson and Carr[13] produced polyneuritis in hens by feeding polished rice, and reported wide variations in the level of blood sugar.

In addition to the sugar analyses, a few isolated determinations seem worthy of note. Thus Kon and Drummond[11] obtained calcium values of 8.2 to 8.4 mgm. per cent in the blood of normal pigeons, and larger amounts, 10.1 to 12.1 in neuritic pigeons. Thompson and Carr[13] reported that polyneuritis has no definite effect on the percentages of creatinine and of non-protein nitrogen in the blood. Uric acid showed a distinct tendency to rise as polyneuritis developed.

Although there is no certain chemical method for diagnosing a deficiency of either of the vitamins A or B, there is abundant evidence that rickets is accompanied by easily determined chemical changes. Bethke, Steenbock, and Nelson[14] suggested that the ash content of the bones, and the calcium and phosphorus content of the blood, would furnish a reliable diagnosis of rickets in rats. They found the blood calcium and phosphorus to be lower during this disease than in the normal rat. The administration of cod liver oil, or exposure to ultraviolet rays, caused these mineral elements to rise to a higher level. Similar observations by Steenbock, Hart, Jones, and Black[15]were made on the chick. Thus in normal chicks the blood calcium varied from 16.93 to 22.57 mgm. per 100 cc. of serum. Corresponding phosphorus values ranged from 6.90 to 9.20 mgm. per 100 cc. In rachitic chicks the range was 9.7 to 16.0 mgm. for calcium, and 3.58 to 4.87 for phosphorus.

Hart, Halpin, and Steenbock[16] reported the inorganic phosphorus in the blood of chicks not receiving cod liver oil as 1.40 to 2.80 mgm. per 100 cc. of serum. The blood of chicks receiving cod liver oil was higher, 2.50 to 5.15 mgm. per 100 cc. of serum.

Hughes and Titus[17] obtained 12.61 mgm. calcium, and 4.57 mgm. per cent inorganic phosphorus in the blood of normal chicks. In rickets these values dropped to 11.14 and 2.95. Ackerson, Blish, and Mussehl[18] published similar results. Their averages of normal birds are 10.61 mgm. per cent for calcium, and 4.60 for phosphorus. In rachitic chicks these values were somewhat reduced, to 7.49 and 3.91 mgm. per cent.

In addition to the analyses just cited, a few more are included as having some value in establishing normal levels of a few of the constituents of chick blood. Sheard and Higgins[19] found, on the average, 12 mgm. calcium, and 6 mgm. phosphorus per 100 cc. of chick serum Cassidy, Dworkin, and Finney[20] gave 200 mgm. per cent as the norma

value of sugar in blood of the chick. Higher values were given by Schwarz and Heinrich,[21] 212 to 309 mgm. per cent. Over half of their determinations fall between the limits, 236-275 mgm. Folin and Denis[22] reported 32 mgm. per cent for non-protein nitrogen, 8 for urea nitrogen, and 4.9 for uric acid. Benedict[23] obtained 4.8 mgm. of uric acid per 100 cc. of blood.

Thompson and Powers[24] made partial analyses of the blood of normal mature hens. The extremes in mgm. per cent for non-protein nitrogen were 33.3 to 85.7. For creatinine 1.14 to 2.78, and for uric acid, 1.09 to 4.86. In the molting stage the levels were raised. Thus for non-protein nitrogen the range was 40.0 to 146.3; for creatinine, 1.77 to 4.05; for uric acid, 1.31 to 5.95.

Numerous mineral analyses of rat bones have been published, but few such determinations on chick bones are available. Steenbock, Hart, Jones, and Black[15] reported the ash content of bones of rachitic chicks as varying from 48.9 to 52.2 per cent. Older chicks, receiving cod-liver oil, had bones with an ash content of 54.6 to 60. 2 per cent. In a later report Hart, Steenbock, and Lepkovsky[25] found that the ash content of the tibias of rachitic chicks, 5-6 weeks of age, was, on the average, approximately 40 per cent. The corresponding value for chicks receiving cod liver oil ranged between 45 and 50 per cent.

EXPERIMENTAL

White Leghorn Chicks have been used exclusively, and cared for as previously described[1]. In each series of observations, except on chicks receiving synthetic rations, there were four groups. One group received an adequate ration, while each of the others received a ration deficient in one of the vitamins, either A, B, or D. The rations themselves are described in Table 1.

In order to be sure that the dietary inadequacies were not unnecessarily complicated with starvation, we attempted to devise rations that are satisfactory in all respects other than the specific deficiency under consideration. That this effort was reasonably successful may be seen by examining the body weights in Tables 7-13. The chicks on the control ration grew rapidly, and were entirely normal in appearance. The analytical results obtained are shown in Tables 2 and 9.

As regards vitamin A, we have had some difficulty in rearing chicks on rations deficient in this factor. In many cases acute symptoms would develop, and the birds die before samples could be obtained for analysis. The normal course of this disease in our experience may be illustrated

TABLE 1.—COMPOSITION OF EXPERIMENTAL RATIONS

Components of Rations	Ration No.							
	503[1]	634[2]	752[3]	753[4]	754[5]	755[6]	830[7]	831[8]
Yellow Corn			81		81		76	
White Corn				81				
Hominy Grits						81		78
Wheat		55.6						
Sodium Chloride		0.9	1	1	1	1	1	1
Calcium Carbonate		1.3	2	2	2	2	2	2
Yeast	15	12.0	6	6	6	4	6	4
Casein	20	12.3	10	10	10	10	10	10
Cod Liver Oil	5	3.0				2	5	5
Lard	10							
Corn Starch	43							
Cellulose	3							
Salt Mixture	4							
Alfalfa Meal		2.5						
Butter Fat		4.2						
Whole Milk Powder		8.2						

[1]Typical of our synthetic rations.
[2]This ration sustains rapid and continuous growth. See Table IX.
[3]Control for the rations deficient in vitamins. The chicks were irradiated daily with a quartz mercury arc, and were given skim milk to drink.
[4]Deficient in vitamin A. The chicks were treated with ultraviolet rays, and were given skim milk to drink.
[5]Deficient in vitamin D. The chicks were given skim milk to drink.
[6]Designed to produce polyneuritis. After 28 days the yeast was removed entirely and replaced by an equal quantity of hominy grits. Slight modifications of this ration were employed in the earlier trials, but they gave essentially the same result, and a detailed description seems unnecessary.
[7]Control for ration 831. These chicks received skim milk to drink. See Table 5.
[8]Deficient in vitamin B. After 28 days yeast was withdrawn from the ration entirely. See Table 6.

by an example. In one group of 9 chicks, a case of leg weakness developed on the 13th day, and all chicks of the group were given the control ration, for one day only. This gave temporary relief, but by the end of the 4th week two chicks had died, and two days later all the others were obviously suffering from a deficiency of vitamin A. The control ration was supplied again, for one day, and the chicks recovered. The symptoms reappeared in an aggravated form by the 70th day, and then the analyses were begun. Our data appear in Tables 3 and 10.

Our experience agrees with that of Nelson, Lamb, and Heller[26] that chicks rarely develop xerophthalmia on diets deficient in vitamin A. Under certain conditions, however, as reported by Beach[27], xerophthalmia develops readily.

The chicks receiving rations deficient in vitamin B grew most slowly, as is to be expected. Somewhat to our surprise, however, evidence of a specific deficiency was very slow to develop. Our observations were under way before it had been demonstrated that vitamin B, as the term was formerly used, is a mixture. We regard our ration as relatively free from any components of the vitamin B complex. The first symptom noted, other than retarded growth, was a form of leg weakness. The chicks were at first disinclined to stand. Later they were unable to stand and within three or four days they seemingly had no control of their legs. Acute symptoms of polyneuritis, with head retraction, were observed in fewer than 50 per cent of the cases. A summary of our observations on vitamin B appears in Tables 4, 5, and 11.

The rachitic group grew very well for about 6 weeks, though leg weakness was observed as early as the fifth week. The chicks were then too small, however, for our purpose and were treated once for 15 minutes with ultraviolet rays. The symptoms then disappeared and usually did not reappear until about the 13th week. By this time most of the chicks had ruffled feathers and crooked legs, and were less inclined to stand unless disturbed. Our analyses are given in Tables 6 and 12.

The purpose of making the analyses here reported was, if possible, to obtain some indication of the cause of the nutritional failures of chicks reared on synthetic diets. Accordingly, a number of these individuals were used for blood and bone analyses. The results are given in Tables 7, 8, and 13.

In collecting material for analysis, an attempt was made to secure samples representative of various ages. Blood was drawn from individual chicks when possible and analysed separately, but if the chicks were too small to yield an adequate sample, composite samples of a group, or part of a group, were analysed. In a few of the earlier analyses blood was obtained by severing the jugular vein. This procedure gave values for inorganic phosphorus that were too high so the heart-puncture method was adopted. Sodium citrate was usually the blood anticoagulant. The procedures used in the blood analyses were as follows: Glucose, by the Shaffer-Hartman[28] method; urea and non-protein nitrogen, according to the Folin-Wu analytical scheme[29]; uric acid, by the Benedict method[30]; phosphorus, according to Briggs' modification of the Bell Doisy method[31]; calcium, by the method of Clark and Collip[32]; with a slight modification. This consisted in precipitation of the calcium oxalate from the trichloracetic acid filtrate of blood, instead of from serum. In addition to the analyses just mentioned, there were also a few deter-

TABLE 2.—BLOOD ANALYSIS OF NORMAL CHICKS[1]

No. of Chick	Age	Calcium	Phosphorus	Glucose	Nonprotein Nitrogen	Urea Nitrogen	Uric Acid
	days	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
781	52	----	4.2	---	---	---	---
778, 779, 782, 780	63	13.0	4.6	190	38.1	5.5	4.4
778, 779, 782, 780	70	12.0	6.0	170	39.9	3.8	4.9
1026	74	12.1	4.6	181	36.6[2]	5.2	5.7
7032	79	14.3	4.6	187	47.6	4.7	3.7
1040	84	11.5	5.5	175	47.1	3.7[2]	4.3
7077, 7052	90	14.0	5.6	204	36.9	2.5	5.0
1041	93	10.7	4.4	197	38.0	4.7	3.5
7052	97	----	6.4	150	47.5	2.3[2]	6.6[2]
807, 838	105	11.3	7.2	220	45.3	5.8	4.5
808	119	11.5	5.5	210	45.6	4.0	4.3
807	140	13.6	4.7	169	67.1	3.2	4.9
1043	142	----	5.6	157	48.8	5.5[2]	5.4
Average	---	12.4	5.3	184	44.9	4.2	4.8

[1]These chicks received Ration 752.
[2]Single determination.

minations[29] of total, and of preformed creatinine. The bones were prepared by the method of Steenbock, Hart, Jones, and Black[15], and the results are reported on the lipoid-free, oven-dry basis. Our analytical results are grouped in the following tables, Nos. 2-13.

TABLE 2a.—TOTAL CREATININE, AND PREFORMED CREATININE IN THE BLOOD OF NORMAL CHICKS.

No. of Chick	Age	Total Creatinine	Preformed Creatinine
	days	mgm. per cent	mgm. per cent
1004 } 1005 }	39	5.1	1.3
1006 } 1008 }	39	4.6	2.1
1010 } 1012 }	39	5.4	1.1
1013 } 1014 }	39	6.7	1.1
1017 } 1021 }	39	4.9	12.
1023 } 1024 }	39	4.6	1.1

[1]These chicks received a practical poultry ration.

TABLE 3.—BLOOD ANALYSES OF CHICKS RECEIVING A RATION[1] DEFICIENT IN VITAMIN A.

No. of Chick	Age	Calcium	Phosphorus	Glucose	Non Protein Nitrogen	Urea Nitrogen	Uric Acid
	days	mgm. per cent	mgm. per cent	mgm per cent	mgm. per cent	mgm. per cent	mgm. per cent
1045	74	15.0	5.9	151	45.9	6.0	7.2
7077, 7022	79	14.5	4.1	185	42.2	3.5	4.2
7094, 7007	79	12.0	4.3	181	50.0	3.0	5.2
7000	79	13.5	4.8	189	40.2	2.7	5.7
7071, 7033	79	15.0	4.0	174	47.8	4.0	5.7
1031	84	12.3	6.4	194	46.5	4.7	5.7
1046	84	11.6	4.3	169	45.8	3.8	3.2
1030	84	11.8	5.3	185	42.9	3.9	4.0
1048	84	13.0	7.8	152	31.1	2.5	4.2
835	119	13.0	4.9	196	40.6	5.1	4.5
Average		13.2	5.2	197	43.3	3.9	5.0

[1]Ration No. 753.

TABLE 4.—BLOOD ANALYSES OF CHICKS RECEIVING A RATION[1] DEFICIENT IN VITAMIN B.

No. of Chick	Age of Chick	Received yeast-free Ration	Calcium	Phosphorus	Glucose	Non-Protein N	Urea N	Uric Acid
	days	days	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
792	70	14	----	---	---	29.4	---	3.4
998[2]	79	20	15.0	4.0	397	65.0	3.0	4.5
7066[2]	80	21	----	---	203	2.2	2.3	4.9
789, 791 794	80	24	12.8	2.2	166	41.2	5.7	1.5
941[2]	82	23	---	----	410	40.6	4.3	4.1
7067, 7042	90	31	14.3	6.7	183	33.2	5.7	4.9
7064[2]	97	38	----	6.2	250	41.0	2.8	5.8
7046	97	38	----	5.5	207	44.8	3.5	4.4
941[2]	97	38	----	6.5	357	70.8	---	6.2
841[2]	105	35	12.6	5.6	320	36.6	---	4.7
842	140	70	12.3	2.3	160	54.0	3.3	2.3
1037	143	71	----	5.3	152	67.8	4.3	4.6

[1]Ration No. 755. This ration was supplied until the chicks were large enough for analysis; the yeast was then removed and replaced by an equal quantity of hominy grite. The period on the yeast-free ration, before the analyses were made, is indicated in the third column.
[2]Chicks had acute symptoms of polyneuritis with head retraction.

TABLE 5.—LEVEL OF BLOOD SUGAR IN CHICKS THAT RECEIVE RATIONS DEFICIENT IN VITAMIN B.

Age	Control Chicks Ration No. 830			Experimental Chicks Ration No. 831		
	No. of Chick	Weight	Blood Sugar	No. of Chick	Weight	Blood Sugar
days		grams	mgm. per cent		grams	mgm. per cent
43	7172	260	231	7180[1]	140	325
44	7163	275	256	8179[1]	130	241
45	7173	280	227	7181[1]	115	218
47	7162	300	220	7175[2]	100	283
48	7169	325	213	7182[2]	190	308
				7178[2]	180	327
50				7183[2]	108	213
52	7162	290	223	7176[2]	135	237
63	7169	420	184			
84	7166	840	199	7184[2]	140	133
86	7163	730	181	7168[2]	260	182
				7173[2]	460	27
90	7166	---	177	7177[3]	110	191
90	----	---	---	7162[3]	400	179
90	----	---		7164[3]	---	178
90	----	---	---	7164[3]	295	178
90	----	---	---	7165[3]	370	179
90	----	---	---	7167[3]	430	200
90	----	---	---	7172[3]	280	185

[1]Blood drawn during acute attack of polyneuritis.
[2]Leg weakness, no symptoms of acute polyneuritis.
[3]This chick received Ration 830 for 53 days, and then Ration 831 for the remainder of the period

TABLE 6.—BLOOD ANALYSES OF CHICKS RECEIVING A RATION[1] DEFICIENT IN VITAMIN D.

No. of Chick	Age	Calcium	Phosphorus	Glucose	Non Protein Nitrogen	Urea Nitrogen	Uric Acid
	days	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
796, 797, 798, 799	53	11.8	2.7	200	37.7	4.6	2.1
796, 797, 798, 799	77	11.1	3.8	167	40.6	3.5	4.4
7020, 7039	90	12.0	3.1	164	32.2	2.4	5.9
7012, 7026	90	10.0	2.1	252	35.8	5.5	7.2
1054	93	9.1	1.5	219	47.4	3.1	5.0
1055	93	9.9	3.2	193	38.6	3.1	6.1
1056	93	8.6	2.4	200	46.6	3.0	5.6
1051	98	9.9	2.2	190	50.0	3.4	4.7
1053	98	10.5	3.7	158	37.8	3.2	4.3
1057	98	11.3	3.9	176	37.5	1.9	4.3
1058	98	10.5	2.7	213	51.5	2.4	5.0
845, 849	115	10.1	3.2	150	38.9	3.8	3.2
Average		10.4	2.9	190	41.2	3.3	4.8

[1]Ration No. 754.

TABLE 7.—BLOOD ANALYSES OF CHICKS RECEIVING SYNTHETIC DIETS[3]
(Glucose and Nitrogenous Constituents).

No. of Chick	Age	Weight of Chick	Glucose	Non-Protein Nitrogen	Urea	Uric Acid	Total Creatinine	Preformed Creatinine
	days	gms.	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
1031, 1034[2]	25	---	226	41.8	---	5.4	4.1	1.4
1029, 1036[1]	25	---	254	49.8	---	6.0	3.9	1.4
1038, 1040 1044[2]	29	---	268	64.0	---	6.9	5.1	1.4
965[1]	34	100	275	--	---	3.3	---	---
928[1]	34	200	229	---	---	4.1	---	---
919[1]	40	390	203	45.0	---	6.6	---	---
989, 990[2]	42	---	245	54.1	---	4.4	5.6	1.2
985, 986[2]	42	---	227	57.5	4.0	4.0	4.2	1.3
988, 992[1]	42	---	220	54.7	---	5.3	4.1	---
920[2]	42	350	269	63.8	---	---	---	---
929[2]	42	298	265	42.6	---	4.4	---	---
959[2]	58	115	174	---	---	5.3	---	---
960[2]	58	130	240	50.0	---	6.8	---	---
962[2]	58	463	249	49.0	3.6	6.0	---	---
963[2]	58	475	196	59.2	3.9	5.2	---	---
926[2]	60	590	255	39.8	5.5	4.6	---	---
957[2]	60	470	227	55.5	6.6	6.5	---	---
961[2]	60	510	222	54.3	4.8	6.9	---	---
918[2]	64	650	216	45.6	7.0	6.4	---	---
921[2]	64	520	201	43.5	5.7	5.7	---	---
924[2]	64	570	192	39.6	---	6.3	---	---
922[2]	71	705	221	51.5	4.1	5.9	---	---
925[2]	71	570	219	45.6	4.9	5.6	---	---
927[2]	71	650	214	49.5	4.4	5.1	---	---
921[2]	74	520	---	--	---	---	4.0	1.3
922[2]	74	705	---	---	---	---	4.2	1.3
925[2]	74	570	---	---	---	---	4.0	1.8
926[2]	74	590	---	---	---	---	4.1	1.4
927[2]	74	650	---	---	---	---	4.3	1.3
961[2]	74	510	---	---	---	---	4.0	1.3

[1]Leg weakness.
[2]No abnorml symptoms except slow growth.
[3]The rations were similar in composition to No. 503, Table 1.

TABLE 8.—BLOOD ANALYSES OF CHICKS RECEIVING SYNTHETIC DIETS.
(Glucose, Calcium, and Phosphorus.)

No. of Chick	Age	Calcium	Phosphorus	No. of Chick	Weight	Age	Glucose
	days	mgm. per cent	mgm. per cent		grams	days	mgm. per cent
				1025[3]	130	11	203
455, 465, 466[1]	25	15.5	5.2	1028[1]	130	20	222
3456, 4052[1]	40	14.4	6.2	1041[3]	115	21	278
3509[1]	40	9.3	6.8	1070[3]	180	25	217
447[3]	45	16.0	3.6	1073[3]	165	25	146
490[2]	45	18.0	4.3	1100[3]	140	25	235
Composite[2]	45	13.2	6.0	1068[3]	150	27	201
Composite[2]	45	15.2	4.5	981[1]	105	28	311
Composite[2]	45	14.0	5.1	982[1]	132	28	205
Composite[2]	45	14.0	4.8	984[1]	90	28	195
Composite[2]	45	12.5	4.6	1045[3]	150	28	237
Composite[2]	56	14.5	7.0	1124[3]	127	28	202
557[2]	56	13.0	6.3	987[3]	215	29	228
487, 489[2]	56	14.4	7.3	1062[1]	210	29	193
442, 443 445[1]	57	13.8	4.5	991[3]	240	32	225
1014[1]	64	14.2	5.2	1049[1]	160	33	230
1015, 1017 1018, 1019[2]	64	12.9	4.7	1050[1]	340	33	217
				1051[1]	250	33	234
1008, 1011[2]	64	12.0	4.5	1053[1]	280	33	226
3458[3]	79	13.8	6.4	1059[1]	220	33	230
				1077[1]	300	33	243
				983[3]	220	34	149
				1054[2]	220	39	234
				1055[1]	170	39	239
				1056[2]	450	39	255
				1057[2]	410	39	242
				1061[2]	390	39	207
				1063[2]	300	39	232
				1102[2]	320	39	222
				1191[2]	325	39	217

[1]Leg weakness. [2]No abnormal symptoms except slow growth. [3]Polyneuritis.

TABLE 9.—BONE ANALYSES OF NORMAL CHICKS

No. of Chick	Weight	Age	Ash	Calcium	Phosphorus
	gms.	days	per cent	per cent	per cent
1[1]	lost	14	37.5	----	----
2[1]	lost	14	43.9	----	----
3[1]	lost	14	39.	----	----
4[1]	lost	14	41.6	----	----
5[1]	120	28	43.0	----	----
7[1]	115	28	44.1	----	----
8[1]	190	42	48.5	18.0	----
9[1]	250	42	47.6	18.8	----
516[2]	590	56	47.0	16.0	7.4
992[2]	555	56	44.9	16.4	8.4
993[2]	495	56	48.6	17.9	8.9
995[2]	655	56	48.2	18.0	----
12[2]	800	63	50.3	18.6	----
1236[2]	830	63	50.6	----	----
1277[2]	920	70	48.8	18.0	9.7
1249[2]	810	70	49.3	18.5	9.6
10[1]	620	70	49.2	18.0	----
781[4]	864	73	48.6	16.9	9.3
780[4]	670	77	50.5	17.0	9.1
778[4]	705	77	50.7	17.8	9.3
779[4]	660	77	48.6	17.1	9.1
782[4]	760	97	47.1	16.8	8.4
2588[3]	780	87	54.2	18.1	10.3
1026[4]	1172	91	49.2	18.0	9.1
1040[4]	890	94	49.8	18.1	9.1
7052[4]	960	103	53.6	19.7	9.5
7077[4]	1020	103	51.1	18.9	9.7
7032[4]	1100	104	55.2	20.8	10.1
1041[4]	990	11	53.3	19.7	9.9
1027[4]	830	123	51.2	19.4	10.9
808[4]	1600	147	59.8	24.6	10.7
1043[4]	1350	149	58.8	20.5	10.4
807[4]	1215	150	51.3	18.4	8.9
838[4]	1410	161	58.4	22.0	10.6

[1]Obtained from University Poultry Farm.
[2]Reared in laboratory on Ration 634.
[3]Reared in laboratory on ration similar to 634.
[4]Reared in laboratory on Ration 752.

TABLE 10.—BONE ANALYSES OF CHICKS RECEIVING RATION[1] DEFICIENT IN VITAMIN A.

No. of Chick	Weight	Age	Ash	Calcium	Phosphorus
	gms.	days	per cent	per cent	per cent
836	187	44	48.0	18.9	9.4
833	205	44	50.4	19.1	9.7
1045	490	77	49.0	18.0	8.9
7071	560	80	50.1	18.1	9.3
7022	600	80	50.9	19.0	10.1
1030	500	91	49.1	18.0	9.9
1048	820	91	50.4	18.0	10.9
1046	630	91	51.0	18.8	9.3
1031	430	91	45.2	----	----
7033	370	96	49.3	17.8	9.5
7074	550	98	50.7	----	----
7000	890	100	54.1	20.7	12.5
7077	440	109	52.3	18.9	9.4
835	725	120	51.7	19.7	9.8

[1]Ration No. 753.

TABLE 11.—BONE ANALYSES OF CHICKS RECEIVING RATION[1] DEFICIENT IN VITAMIN B.

No. of Chick	Weight	Age	Ash	Calcium	Phosphorus
	gms.	days	per cent	per cent	per cent
792	206	70	46.2	16.4	8.3
790	285	75	42.2	16.1	7.5
998	390	79	47.5	17.5	8.7
791	305	80	37.9	14.2	----
789	390	80	49.1	17.0	8.5
794	470	80	54.0	18.0	----
7066	210	81	49.5	19.1	8.9
7067	200	100	40.7	16.0	7.3
841	570	105	44.8	----	6.7
941	215	107	45.7	17.6	8.6
7046	510	107	52.4	19.5	9.7
7064	370	109	48.1	17.8	8.7
1037	620	138	53.2	19.3	9.4
842	760	148	45.7	17.6	8.5

[1]Ration No. 755.

TABLE 12.—BONE ANALYSES OF CHICKS RECEIVING A RATION[1] DEFICIENT IN VITAMIN D.

No. of Chick	Weight	Age	Ash	Calcium	Phosphorus
	gms.	days	per cent	per cent	per cent
797	400	63	38.3	13.5	6.7
795	440	70	36.6	15.8	7.4
798	533	70	41.3	14.6	6.1
796	550	70	37.6	12.5	6.9
799	460	70	40.8	15.6	7.9
1058	560	98	44.1	14.2	7.7
7039	850	101	45.6	16.6	7.9
7026	875	102	47.3	----	----
012	560	102	41.2	14.7	7.7
7020	540	102	44.4	15.1	7.9
1053	700	111	46.9	----	----
1054	790	111	42.8	15.5	7.8
1057	650	111	46.1	15.9	7.9
1051	900	112	42.7	15.1	7.8
1056	940	113	44.6	16.0	7.9
1055	750	114	44.5	17.4	8.3
800	518	148	43.6	14.5	7.6
848	1350	148	56.6	19.1	10.4
845	960	148	54.4	17.0	10.2
847	1030	161	59.0	19.8	10.9

[1]Ration No. 754.

TABLE 13 —BONE ANALYSES OF CHICKS RECEIVING SYNTHETIC DIETS[3]

No. of Chick	Weight	Age	Ash	Calcium	Phosphorus
	gms.	days	per cent	per cent	per cent
453[1]	65	21	45.2	16.3	10.8
468[1]	85	21	43.2	16.7	9.1
471[2]	90	21	41.3	15.8	8.3
465[1]	90	21	40.3	15.2	8.2
466[1]	60	21	41.0	15.0	8.9
455[1]	58	21	36.0		8.4
476[1]	110	28	41.5	16.8	8.1
474[1]	95	28	44.8	17.8	9.6
107[1]	60	30	48.7	----	----
109[1]	64	30	47.7	----	----
108[1]	62	31	47.7	----	----
3456[1]	88	40	43.6	----	----
3509[1]	110	40	47.6	----	----
3480[1]	100	40	46.1	----	----
447[3]	120	42	48.6	18.2	9.1
490[2]	365	42	48.8	18.8	9.0
565[2]	300	42	46.1	18.2	8.5
539[2]	105	42	49.6	18.2	9.9
543[3]	160	42	46.6	18.8	9.3
540[1]	80	42	45.4	18.5	9.4
579[2]	130	42	45.5	18.0	9.8
578[1]	120	42	48.7	19.5	9.0
568[2]	180	42	39.5	15.0	7.8
567[1]	180	42	47.4	18.9	10.7
571[1]	108	42	46.9	17.6	9.1
545[2]	215	42	40.9	15.9	8.9
546[1]	90	42	48.1	18.5	9.8
583[2]	360	42	43.2	16.0	7.8
430	56	56	55.4	----	8.3
488[2]	345	56	43.6	20.5	----
555[2]	652	56	48.8	16.7	----
554[2]	480	56	49.9	----	----
557[2]	200	56	59.8	----	----
442	56	56	42.4	18.5	9.4
445[2]	120	56	49.5	18.4	9.7
443[2]	375	56	46.0	17.2	8.5
1008[2]	600	70	50.1	----	----
1019[1]	650	70	49.8	----	----
1014[1]	750	70	51.0	----	----
356[1]	360	79	46.6	----	----

[1]Leg weakness.
[2]No abnormal symptoms except slow growth.
[3]Polyneuritis

Inspection shows that our determinations are in essential agreement with those of other workers using the newer methods, so extended discussion is unnecessary. It is evident that chicks on rations deficient in vitamin A do not develop abnormalities in either blood or bone that can be detected by the analyses we made. In regard to vitamin B, a high value for blood sugar has been reported by several workers. Of the eleven glucose determinations described in Table 4 (deficient in vitamin B), five did not develop the acute symptoms so characteristic of polyneuritis, and not one of these had an abnormally high blood sugar. Of the six analyses made on chicks with acute symptoms, five were abnormally high, with a range of 250 to 410 mgm. per cent. In Table 5 a special study was made of the blood sugar in chicks on rations deficient in vitamin B. The controls gave higher values than usual for blood sugar, with one exception, but the range was markedly lower than among the birds receiving the inadequate diet. Of the latter group, 11 developed obvious symptoms of vitamin B deficiency. Of these, 7 gave high values for blood sugar, 237 to 327 mgm. per cent. The other 4 were normal, and one of them was unusually low in this constituent, 133 mgm. per cent. Of the six chicks that had been transferred to the control ration, and analyzed at 90 days of age, not one had an abnormally high content of blood sugar.

Of the 9 determinations of inorganic phosphorus in the blood (Table 4), 2 were so low as to suggest rickets. Since the ration of these chicks contained 2 per cent of cod liver oil, we thought we had provided ample protection against a deficiency of vitamin D. One of these chicks, No. 842, was killed somewhat later and the femur analysed. The ash content was slightly low. In the other case the blood sample was a composite from 3 chicks, Nos. 789, 791, and 794. The ash content of the femur of one of these was low, the other two were normal.

Of the chicks receiving rations deficient in vitamin D, the analyses are characteristic of rickets. The blood phosphorus was about half the normal value, and the blood calcium was about 15 per cent lower than in normal chicks. The ash content of the bones was reduced, but extremely low values were not obtained. Three of the chicks, killed at ages of 148 days for two, and 161 for the other, had normal values. The ash content is sufficiently high to suggest that the leg weakness observed was not necessarily due to structural weakness of the bones.

Our original purpose in making the analyses reported in this paper was to determine whether or not the inadequacy of our synthetic rations is associated with any of the known deficiency diseases. The analyses alone are of little value in detecting a deficiency of vitamin A, for only normal values were obtained in chicks reared on rations known to be

deficient in that vitamin. As regards vitamin D, the determination of inorganic phosphorus in the blood was sufficient of itself to indicate that rickets was not to be considered. Such a conclusion is confirmed by the analyses of bone.

In regard to vitamin B, our conclusions are less final. In our experience the blood sugar of normal chicks is usually under 200 mgm. per cent. Thus of 23 normal chicks analysed, all but 8 were lower than 200. If we include with these normal chicks, all those analysed that received rations deficient in either vitamin A or D we have 47 in all. Of these, only 13 had blood sugar values of 200, or more, mgm. per cent. Tables 7 and 8 include 54 analyses of blood sugar from chicks reared on synthetic rations. Of these, 47 were 200 mgm. per cent, or higher. We have decided, therefore, that though the evidence of a deficiency of vitamin B is not conclusive, such a possibility is clearly indicated. It was noted in another publication[33] that many chicks receiving synthetic diets had enlarged adrenal glands, and this is also interpreted as evidence of a deficiency of vitamin B. If this proves to be the case, the data as yet give no indication of the number of factors in the vitamin B complex, or as to which are lacking in our synthetic diets.

If there are sex differences in the composition of either blood or bone, our data are insufficient to establish such a fact. For that reason the sex of the experimental animals has not been indicated.

It seems desirable to include at this time brief mention of blood counts taken, though they were not helpful in explaining the nutritional failures. It seemed possible that the poor condition of the chicks was related to anemia, so red cell counts were made on a number of individuals. They were from 3 to 7 weeks of age, and the sexes are not separated, as in most cases we were not able to distinguish between them. Some were quite normal in appearance, others had marked symptoms of leg weakness. These were divided into groups, according to their condition, and the blood counts compared. These were quite variable, the extremes ranging from 2,000,000 to 4,000,000 per cmm., but the averages of the two groups were paractically identical, a little less than 2,940,000 erythrocytes per cmm. For purposes of comparison we made a few counts on mature chicks from the University Poultry Farm. The average of the cocks was 3,323,000 per cmm., of the hens 3,067,000. Fritsch[34] gives 3,240,000 for cocks, and 2,770,000 for hens.

SUMMARY

Chicks reared on synthetic diets usually develop symptoms of a deficiency disease. In an effort to define more precisely the nature of the deficiency, chicks reared on such rations were used for the analysis

of blood and bone. These analyses were then compared with similar determinations made on normal chicks, and on chicks receiving rations deficient in one of the following vitamins, A, B, or D.

The analyses of chicks receiving rations deficient in vitamin A were normal. A ration deficient in vitamin B apparently raised the level of blood sugar. A deficiency of vitamin D lowered the inorganic phosphorus of the blood, and the ash content of the bones. The blood sugar of chicks reared on synthetic diets was high so we have adopted the tentative hypothesis that these rations are deficient in one or more factors of the vitamin B complex.

ACKNOWLEDGMENT

The writer takes this opportunity to express his appreciation of the assistance and criticisms received from various sources while the studies here reported were in progress. He is especially indebted to Prof. A. G. Hogan and the other members of the Department of Agricultural Chemistry, and to the staff of the Department of Poultry Husbandry.

BIBLIOGRAPHY

1. Hogan, A. G., Guerrant, N. B., and Kempster, H. L., 1925. Mo. Agr. Exp. Sta. Res. Bul. 81.
2. Osborne, T. B., and Wakeman, A. J., 1919. Jour. Biol. Chem. xl, 383.
3. Ungar, A., 1927. Biochem. Zeitschr. clxxx, 357.
4. Funk, C., and v. Schonborn, E. 1914. Jour. Physiol. xlviii, 328.
5. Funk, C. 1920. Jour. Physiol. liii, 247.
6. Ogata, C., Kawakita, S., Oka, C., and Kagoshima, S. 1921. Abs. in Exp. Sta. Record. xlvi, 668.
7. Redenbough, H. E., 1927. Proc. Soc. Exp. Biol. and Med. xxiv, 842.
8. Randoin, Mme. L., and Lelesz, M. E. 1925. Compt. Rend. Acad. Sci. clxxx, 1366.
9. Barlow, O. W. 1927. Amer. Jour. Physiol. lxxxiii, 237.
10. Collazo, J. A., 1922. Biochem. Zeitschr. cxxxiv, 194.
11. Kon, S., and Drummond, J. C., 1927. Biochem. Jou r. xxi, 632.
12. Marrian, G. F., Baker, L. C., Drummond, J. C., and Wollard, H. 1927. Biochem. Jour. xxi, 1336.
13. Thompson, T. J., and Carr, Ida L. 1923. Biochem. Jour. xvii, 373.
14. Bethke, R. M., Steenbock, H., and Nelson, M. T. 1923. Jour. Biol. Chem. lviii, 71.
15. Steenbock, H., Hart, E. B., Jones, J. H., and Black, A. 1923. Jour. Biol. Chem. lviii, 59.
16. Hart, E. B., Halpin, J. G., and Steenbock, H. 1922. Jour. Biol. Chem. lii, 379.
17. Hughes, J. S., and Titus, R. W., 1926. Jour. Biol. Chem. lxix, 289.
18. Ackerson, C. W., Blish, M. J., and Mussehl, F. E. 1925. Jour. Biol. Chem. lxiii, 73.
19. Sheard, C. and Higgins, G. M. 1928. Amer. Jour. Physiol. lxxxv, 290.
20. Cassidy, G. J., Dworkin, S., and Finney, W. H. Amer. Jour. Physiol. lxxv, 609.
21. Schwarz, K., and Heinrich, K. 1928. Biochem. Zeitschr.cxciv, 346.
22. Folin, O., and Denis, Winifred. 1913. Jour. Biol. Chem. xiv. 29.
23. Benedict, S. R., 1915. Jour. Biol. Chem. xx, 633.
24. Thompson, T. J., and Powers, H. H., 1925. Poultry Science iv, 186.
25. Hart, E. B., Steenbock, H., and Lepkovsky, S. 1925. Jour. Biol. Chem., lxv, 571.
26. Nelson, V. E., Lamb, A. R., and Heller, V. G., 1922. Amer. Jour. Physiol. lix, 335.
27. Beach, J. R., 1924. Cal. Agr. Exp. Sta. Bull. 378.
28. Shaffer, P. A., and Hartman, A. F. 1921. Jour. Biol. Chem. xlv, 349.
29. Folin, O., and Wu, H., 1919. Jour. Biol. Chem. xxviii, 81.
30. Benedict, S. R. 1922. Jour. Biol. Chem. 291.
31. Briggs, A. P., 1924. Jour. Biol. Chem. xlix. 255
32. Clark, E. P., and Collip, J. B., 1925. Jour. Biol. Chem. lxiii, 461.
33. Hogan, A. G., Shrewsbury, C. L., and Kempster, H. L., 1929. Mo. Agr. Exp. Sta. Res. Bulletin 122.
34. Fritsch, G. 1920. Pfluger's Archiv. clxxxi, 78.

BIOGRAPHICAL

The author of this dissertation was born at Livonia, Washington County, Indiana, on October 19, 1898. Upon completion of the course of study in the graded school he entered the High School of Parsons, Kansas. During his senior year he entered the military service. He was given an honorable discharge in 1919, and entered William Jewell College, at Liberty, Missouri. In September, 1921, he was appointed student assistant in Chemistry and held this position until graduation in 1923. The following fall he entered Washington University as a graduate student, and assistant in biological Chemistry. In the spring of 1925 he accepted a position as assistant in Agricultural Chemistry at the University of Missouri, and enrolled in the graduate school that summer.